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# Chronic Stimulation of the Pituitary-Adrenal Axis in Rats by Interleukin-1 $\beta$ Infusion: *In Vivo* and *In Vitro* Studies\*

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**ABSTRACT.** It has been shown that acute administration of interleukin-1 (IL-1) to rats elicits a transitory increase in plasma ACTH and corticosterone (B) levels. To investigate the effects of chronic administration of IL-1 on plasma ACTH and B levels, in the present study rats were equipped with Alzet osmotic minipumps loaded with either IL-1 (delivery rate 0.5, 2.0, or 4.0  $\mu$ g/24 h, ip, for 1 week) or saline. At the end of the treatment the rats were decapitated, the adrenals were weighed, and the *in vitro* release of  $\beta$ -endorphin ( $\beta$ E) by the anterior pituitary and that of B by the adrenal gland were measured. Continuous administration of 2.0 and 4.0  $\mu$ g IL-1/24 h resulted in a persistent increase in plasma ACTH and B concentrations compared to the levels in saline-infused rats, with peak levels on the first day of administration. In addition, adrenal weights of IL-1 rats were significantly higher than those of saline rats. The 4.0- $\mu$ g IL-1/day *in vivo* treatment induced an increase in spontaneous *in vitro* secretion of  $\beta$ E and B, while the *in vitro* responses of the

pituitary (to CRF) and the adrenal (to ACTH) of animals treated *in vivo* with IL-1 were significantly diminished. IL-1 at a dose of 0.5  $\mu$ g failed to affect plasma ACTH and B values, adrenal weight, and *in vitro*  $\beta$ E and B secretion. Chronic infusion of rats with 4.0  $\mu$ g IL-1/day induced prolonged fever, whereas at lower doses of IL-1 (2.0 and 0.5  $\mu$ g), temperatures were elevated only on the first 2 days of infusion. IL-1 at doses of 2.0 and 4.0  $\mu$ g/day induced suppression of body weight gain on the first 2 days of the treatment period compared to saline treatment. Plasma norepinephrine and/or epinephrine concentrations were raised only on day 1 of the 2.0- and 4.0- $\mu$ g IL-1 experiments. Thus, the observed effects of IL-1 on the hypothalamo-pituitary-adrenal axis probably do not result merely from stress induced by the treatment. Taken together, our data show the potential of IL-1 to induce a dose-dependent and long term activation of the pituitary-adrenal axis. (*Endocrinology* 130: 1153–1164, 1992)

**S**UBSTANTIAL evidence has now accumulated that there is a functional relationship between the immune and the (neuro)endocrine system. Interleukin-1 (IL-1), a pleiotropic polypeptide synthesized and released predominantly by macrophages, acts as a primary mediator of the acute phase response to microbial invasion and physical stressors (1, 2). In addition to its role in the coordination of host defense mechanisms, IL-1 is thought to serve as a trigger for the activity of the hypothalamo-pituitary-adrenal (HPA) axis, which is chronically stimulated under these conditions (3–5).

Evidence for this idea is primarily based on *in vivo* experiments in laboratory animals. It has been shown

that acute administration of IL-1 activates the HPA axis in mice and rats, as manifested by increased levels of ACTH and/or corticosterone (B) in plasma (3, 6–8). Whether IL-1 can directly act at the pituitary and/or adrenal level to release ACTH and B, respectively, is still controversial. Strong evidence exists that the brain is the primary site of action, and that IL-1, either directly or indirectly, acts at the level of the hypothalamus by stimulation of CRF release (6, 7, 9–12). In these studies IL-1 was administered as a single bolus injection in relatively high doses, and plasma levels of ACTH and/or B were followed for only a few hours. IL-1 has been shown to induce fever (13), to inhibit food intake (14–16), and, in high doses, to be toxic to the animal (17). Little or no attention has been given in studies on the effects of IL-1 on the HPA axis to the question of whether these effects resulted from a direct action of IL-1 on the HPA axis or were secondary to stress effects induced by IL-1 administration.

Received October 10, 1991.

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\* This work was supported by the Royal Netherlands Academy of Arts and Sciences.



Increased levels of IL-1 and, in particular, of IL-6 and tumor necrosis factor have been measured in biological fluids of patients suffering from infection or inflammatory diseases (18–20). In the present study we investigated whether IL-1 is capable of mediating long term changes in HPA activity. To mimic the setting of chronically elevated IL-1 production that is thought to occur during some infectious diseases, we implanted osmotic minipumps in rats in order to infuse low doses of 0.5, 2.0, and 4.0  $\mu\text{g}$  IL-1/day continuously during a period of 1 week. ACTH and B levels were measured in blood samples, which were withdrawn daily by means of a chronic jugular cannula. In addition, we investigated the *in vitro* secretion of  $\beta$ -endorphin ( $\beta\text{E}$ ) and B from pituitary and adrenal glands of rats chronically infused with IL-1 or saline *in vivo*. To detect stress effects, particular attention was given to the effects of chronic administration of IL-1 on body temperature and food consumption and on plasma levels of the stress hormones PRL, nor-epinephrine (NE), and epinephrine (E). In the present longitudinal study we show that continuous infusion of rats with low doses of IL-1 elicits a long term activation of the HPA axis, and it is evidenced that these effects of IL-1 are not simply secondary to stress effects induced by IL-1 administration.

## Materials and Methods

### Test materials

Recombinant human IL-1 $\beta$  (rhIL-1 $\beta$ ) was kindly provided by Dr. D. Boraschi (Sclavo, Siena, Italy). The preparation has a specific activity of  $10^9$  U/mg protein on D10.G4.1 cells, corresponding to  $10^5$  U/ $\mu\text{g}$  vs. the interim IL-1 $\beta$  reference reagent 86/552. According to the specifications of the suppliers, endotoxin contamination was negligible ( $<1.2$  ng lipopolysaccharides/mg IL-1). rhIL-1 $\beta$  was diluted in sterile pyrogen-free saline.

### Animals

Male albino Wistar rats (Cpb:WU) were obtained from the local breeding facility. They were individually housed in Plexiglass cages in an artificially lighted room (lights on at 0700 h; lights off at 1900 h). Rats were provided commercial rat chow (RMH-TM, Hope Farms, Woerden, The Netherlands) and tap water *ad libitum*. At the start of the experiments rats were 10 weeks old and weighed 200–220 g.

### *In vivo* experimental procedures

To diminish the stress caused by the experimental procedures, the animals were handled daily by the experimentator, starting 1 week before cannulation. Body weight was measured daily at 0800 h, and food and water intake was estimated by weighing the residual food and water for individual cages. Body temperature was measured serially twice a day between 0830–0900 h and between 1300–1430 h in conscious hand-held rats by insertion of a thermal probe into the rectum. The probe was

connected to a digital temperature monitor (Digital DT100, Elbatron, Kerkdriel, The Netherlands). The mean daily temperature for each rat was determined by averaging the morning and afternoon rectal temperatures.

Blood was collected from freely moving rats by means of a chronic cannula. Rats were cannulated according to the method described by Steffens (21) with some minor modifications. Briefly, under pentobarbital (60 mg/kg BW, ip; Apharma, Arnhem, The Netherlands)-atropine (0.125 mg/kg, im; Pharmachemie, Haarlem, The Netherlands) anesthesia, a Silastic cannula (id, 0.5 mm; od, 0.94 mm; Dow-Corning Corp., Midland, MI) was inserted into the right external jugular vein and passed down to the atrium. The distal end of the cannula was tunneled sc and exteriorized through a stab wound in the skin on the head, where it was connected to a hooked stainless steel tube. This assembly was anchored to the skull with three stainless steel screws and acrylic cement. During cannulation, rats were continuously exposed to a gas flow of O<sub>2</sub>-N<sub>2</sub>O (each 500 ml/min). The cannula was filled with a 0.9% NaCl solution containing heparin (500 IU/ml; Organon Teknika, Oss, The Netherlands) and polyvinylpyrrolidone (1 g/ml; Merck, Darmstadt, Germany).

Seven to 9 days after cannulation, rats were equipped with osmotic minipumps (1  $\mu\text{l/h}$ ; model 2001, Alzet Corp., Palo Alto, CA), which were loaded with rhIL-1 dissolved in sterile pyrogen-free physiological saline or with saline alone and equilibrated, immersed in saline, for 3–4 h at 37 C according to the instructions of the manufacturer. The pumps were implanted ip in ether-anesthetized animals (1400–1600 h). Three separate experiments, each including 14 animals, were performed, in which 1 group of rats ( $n = 7$ ) was continuously infused at a rate of 0.5, 2.0, or 4.0  $\mu\text{g}$  IL-1/day·rat. In each experiment a control group ( $n = 7$ ) receiving 0.9% pyrogen-free saline-filled osmotic pumps was included. The indwelling cannula and the osmotic pumps were tolerated well by the animals, with no signs of discomfort or infection.

At the end of the experiments, the pumps filled with IL-1 were found to be encapsulated by a firm layer of granulation tissue, with deposition of collagen and abundant fibroblasts and capillaries. Pumps delivering the higher doses of IL-1 (4.0 and 2.0  $\mu\text{g}$  IL-1/day) were more encapsulated than those delivering 0.5  $\mu\text{g}$ /day. Saline-loaded pumps were not encapsulated. Mrosovsky *et al.* (22) elegantly showed that encapsulation of the pumps does not block the release of IL-1 into the peritoneal cavity and that IL-1 under these circumstances remains bioactive for at least 1 week.

**Blood sampling.** Blood was collected once a day for 8 days starting on the morning before implantation of the pumps (day 0 up to day 7), as described by Wiersma and Castelijns (23) with some minor modifications. Because of the circadian variation in hormone release, blood was withdrawn daily from the animals between 1000–1200 h. Blood samples (2.0 ml) for ACTH, B, PRL, E, and NE determinations were collected in prechilled tubes containing dry lithium-heparin additive (30 USP U/tube; Vacutainer, Becton Dickinson, Etten-Leur, The Netherlands). Blood samples were gently shaken and spun for 10 min at  $1500 \times g$  (4 C). Plasma was separated, and red blood cells were resuspended in sterile physiological saline (1.5 ml) and returned



to each rat. For PRL determination, plasma (80  $\mu$ l) was diluted with 160  $\mu$ l PBS, and for catecholamine determination, plasma was stored in tubes containing 0.24 M EGTA (Merck) and 0.2 M glutathione (Sigma, St. Louis, MO). Plasma samples were aliquoted and stored at  $-20^{\circ}\text{C}$  until assayed.

#### *In vitro experimental procedures*

Rats were decapitated 8 days after implantation of the pumps. Immediately after decapitation (1000–1030 h) the brain was removed, the pituitary gland was taken out, and the anterior lobe (AL) was carefully separated from the neurointermediate lobe. The adrenals were removed and placed together with the ALs in a tissue holder containing ice-cold carbogenated superfusion medium.

**Superfusion.** After dissection, the adrenals were freed of fat, weighed, cut in about 16 pieces, and transferred to a superfusion apparatus (2 adrenals of 1 animal/chamber). ALs were quartered and also transferred to the apparatus (1 AL/chamber). The tissues were superfused continuously with carbogenated Krebs-Ringer bicarbonate buffer (118 mM NaCl, 4.85 mM KCl, 1.15 mM  $\text{KH}_2\text{PO}_4$ , 1.15 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 25 mM  $\text{NaHCO}_3$ , and 1.25 mM  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) supplemented with BSA (Sigma fraction V; 0.5%), ascorbic acid (0.1 mM), and glucose (11.1 mM; superfusion medium). The flow rate was kept constant at 0.1 ml/min using a Gilson Minipuls 3 peristaltic pump (Meyvis, Bergen Op Zoom, The Netherlands). The temperature of the superfusion chambers and media was kept constant at  $37^{\circ}\text{C}$ . In all experiments, the tissues were first superfused for 45 min to allow the release of  $\beta\text{E}$  immunoreactivity ( $\beta\text{E-IR}$ ) and B to reach a rather stable level. From then on, fractions (15 min; 1.5 ml) were collected in ice-chilled tubes, and superfusion was continued for 165 min without further experimental manipulations. After this period, the ALs were exposed for 30 min (fractions 12–13) to medium containing  $10^{-9}$  M rat CRF (Byk, Zwanenburg, The Netherlands), and the adrenals for 60 min (fractions 12–15) to medium containing 250 pg/ml ACTH (MRC 74/555). Thereafter, superfusion was continued for another 120 (ALs) or 90 (adrenals) min with the initial medium. One hundred-microliter aliquots of the AL superfusate fractions were taken for direct RIA of  $\beta\text{E-IR}$ , whereas the total adrenal superfusate fractions (1.5 ml) were taken for B determination.

**Calculations.** The spontaneous rate of release of  $\beta\text{E-IR}$  and B was not identical for the different ALs or adrenals within the same experiment. To compare results between the superfusion chambers, the total amount of drug-induced  $\beta\text{E-IR}$  (fractions 12–17) or B (fractions 13–19) release was summated and expressed as a percentage of the calculated basal release. The area under the line linking the means of the  $\beta\text{E-IR}$  or B content of the two fractions immediately preceding ( $\beta\text{E-IR}$ , fractions 10–11; B, fractions 11–12) and the first two fractions in which stabilization of the release to basal levels had occurred ( $\beta\text{E-IR}$ , fractions 18–19; B, fractions 20–21) was taken as basal release.

#### *Hormone extraction and measurement*

**ACTH. Extraction from anterior pituitaries:** The ACTH content of the anterior pituitary was investigated in a separate fourth

experiment. Anterior pituitaries of rats were rapidly dissected on the seventh day of continuous infusion with 2.0  $\mu\text{g}$  IL-1/day or saline. Anterior pituitaries were frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$ . These tissues were heated for 10 min in 1.0 N acetic acid (1:10, wt/vol) in a boiling water bath and subsequently cooled on ice. Then, the tissues were homogenized by sonification and centrifuged at  $20,000 \times g$  for 20 min at  $4^{\circ}\text{C}$ . The supernatants were evaporated to dryness, and the residues were dissolved in 1 ml PBS containing 0.25% BSA and 0.2% sodium azide. Insoluble material was removed by centrifugation, and the supernatants were stored at  $-20^{\circ}\text{C}$ .

**Extraction from plasma:** For measurement of ACTH in rat plasma, 50- $\mu$ l aliquots of plasma sample to each of which 250  $\mu$ l ACTH-free human plasma (pooled from 20 healthy volunteers after suppression of ACTH secretion by dexamethasone) had been added was used. ACTH was extracted and concentrated from tissue extracts and plasma by the addition of 25 mg Vycor glass powder (325 mesh; Corning Glass Works, Corning, NY) in 250  $\mu$ l bidistilled water. The mixture was inverted end over end for 30 min at  $4^{\circ}\text{C}$ . After centrifugation ( $2,000 \times g$ ; 10 min;  $4^{\circ}\text{C}$ ), the supernatants were discarded. The pellets were washed with 2 ml bidistilled water and 2 ml 1 N HCl, respectively. ACTH was eluted from the glass pellet by rotation (30 min at  $4^{\circ}\text{C}$ ) with 1 ml of a mixture of acetone and bidistilled water (50:50, vol/vol). After centrifugation, the acetone fractions were decanted and evaporated to dryness, and the residues were reconstituted in 125  $\mu$ l of a solution containing 0.9% NaCl and 0.25% BSA (ORHD 20/21, Behring, Marburg, Germany), pH 3.5, at  $4^{\circ}\text{C}$  for 60 min to ensure maximal reuptake of the hormone. After centrifugation for 45 min at  $30,000 \times g$ , a 100- $\mu$ l aliquot was taken from this solution for estimation of the ACTH content by RIA. Standard curves were prepared by spiking ACTH-free plasma with ACTH (MRC 74/555). The standard samples were subjected to the same extraction procedure as the unknowns in order to compensate for procedural losses. The recovery was approximately 70%. All samples were extracted in triplicate.

**RIA:** ACTH-IR in the supernatants was measured by RIA, using a commercial ACTH antibody (IgG Corp., Nashville, TN). ACTH-(1–39) was used as standard, and [ $^{125}\text{I}$ ] ACTH-(1–39) as tracer. The ACTH RIA was performed as follows. One hundred microliters of rabbit ACTH antiserum [final dilution, 1:30,000 in ACTH RIA buffer (phosphate buffer containing 13 mM EDTA, 0.02% sodium azide, 0.25% BSA (ORHD 20/21, Behring), 0.1% Triton X-100, and 250 kallikrein inhibitor units aprotonine (Bayer, Leverkusen, Germany)/ml)] were added to 100  $\mu$ l supernatant (sample or standard). The mixture was preincubated for 3 days at  $4^{\circ}\text{C}$  with antiserum. Then, tracer ( $\sim 7000$  dpm/100  $\mu$ l) was added, and incubation was continued for another day. Bound and free ACTH were separated by a second antibody system. One hundred microliters of a separation reagent [10% (vol/vol) sheep antirabbit immunoglobulin G and 0.01% (wt/vol) rabbit immunoglobulin G (Sigma)] were added to each tube and incubated for 20 min at room temperature. The antibody complex was precipitated by the addition of 1 ml 7.5% polyethylene glycol 6000 (Merck). The sensitivity of the assay system was 10–15 pg/ml, and the within- and between-assay coefficients of variation of the ex-



traction and RIA procedure were 9.1% and 15.3%, respectively. **B.** Plasma B was measured by RIA after extraction using an antiserum from our own laboratory (S-05 230676) raised in sheep against a B-21-hemisuccinate-BSA conjugate. The following cross-reactivities (expressed as percentage on a mass basis) were obtained: desoxy-B, 28.6%; cortisone, 0.2%; cortisol, 0.8%; deoxycortisol, 0.3%; testosterone, 3.8%; and progesterone, 22.5%. To each plasma sample (50  $\mu$ l), 100  $\mu$ l 0.1 N NaOH, 100  $\mu$ l B tracer [ $[1\alpha,2\alpha\text{-}^3\text{H}]\text{B}$ ; Amersham International PLC, Amersham, Aylesbury, Buckinghamshire, United Kingdom; 10,000 dpm in 0.2% ethylene glycol (Merck)/water (EGW)] and 500  $\mu$ l bidistilled water were added. Extraction was carried out using 7.5 ml dichloromethane (Baker, Deventer, The Netherlands). The water phase was discarded, and the dichloromethane phase was evaporated. The residue was dissolved in 2 ml 0.2% EGW, and the concentration of B in the eluate was measured by RIA. Briefly, aliquots of 100, 200, or 300  $\mu$ l eluate were transferred to test tubes, and the volume was made up to 300  $\mu$ l with 0.2% EGW and mixed with 200  $\mu$ l of an antiserum dilution (final dilution, 1:100,000) and 100  $\mu$ l B tracer (10,000 dpm/tube). Antiserum and tracer were diluted in 0.05 M borate buffer (pH 8.0) containing 0.1% human  $\gamma$ -globulin (Beriglobin S, Behring, Marburg, Germany). Aliquots of 300  $\mu$ l eluate were taken for recovery. Amounts of 0–5,000 fmol/tube B (Stereoids, Inc., Wilton, NH) diluted in 0.2% EGW were used for obtaining a standard curve. After incubation overnight at 4 C, the suspension was mixed with 150  $\mu$ l dextran-coated charcoal suspension and left at 4 C for 5 min. After centrifugation, the supernatants were decanted into counting vials, and 4 ml Aqualuma (Lumac LSC, Olen, Belgium) was used as counting solution for radioactivity measurements. The sensitivity of the assay was 25–45 fmol/tube. The within- and between-assay coefficients of variation were 10.0% and 16.7%, respectively.

B produced by adrenal glands *in vitro* was measured fluorometrically, as described by Goverde *et al.* (24).

**PRL.** Plasma PRL levels were measured directly by RIA, using reagents [purified rat PRL (AFP-10505B) for iodination; rabbit antirat PRL antiserum (AFP-131581580), and rat PRL reference preparation (AFP-4459B)] obtained from the NIDDK through the courtesy of Dr. Raiti of the National Hormone and Pituitary Program, University of Maryland School of Medicine. The RIA was performed in essence as described by Wiersma (25). The sensitivity of the assay was 160 pg/ml, and the within-assay coefficient of variation was 4.4%. All necessary comparisons between test and control animals were made within one assay run. Data are expressed as picograms of PRL per ml plasma.

**Catecholamines.** Plasma catecholamines (E and NE) were determined by using HPLC, followed by fluorescence detection, as described in detail by van der Hoorn *et al.* (26). Catecholamines were concentrated from plasma by liquid-liquid extraction and derivatized with the fluorescent agent 1,2-diphenylethylenediamine before chromatography. The instrumentation for the chromatography consisted of a 610 Fluid unit pump, a WISP 710 B autoinjector, and a 470 scanning fluorescence detector, all from Waters Associates (Etten-Leur, The Netherlands). All separations were performed on a Nova-Pak C18

column. Calculations were made on a NEC Powermate SX plus computer using the Baseline 815 program (Waters Associates).

**$\beta$ E.** The RIA procedure was performed essentially as described by Sweep *et al.* (27), using an antiserum kindly provided by Dr. V. M. Wiegant, Rudolf Magnus Institute (Utrecht, The Netherlands).

#### Chemicals

All chemicals used were of analytical grade.

#### Statistical analysis

All data are presented as the mean  $\pm$  SE of five to seven rats. Comparisons between IL-1 and saline groups were made by analysis of variance with repeated measurements to analyze the effects of the treatment course. To get a fair picture of the effect of continuous IL-1 treatment, analysis of repeated measures was performed from days 2–7, as on the first day changes occurred in ACTH, B, and catecholamine levels in plasma, and in body temperature, food consumption, and body weight, which in most instances surpass the changes seen from days 2–7. When analysis of variance revealed a statistically significant difference, comparison of the individual groups at specific time points was further evaluated by Student's *t* test, as were the effects of IL-1 on the first day of treatment. For single comparisons between IL-1 and saline groups, Student's *t* test was used.

## Results

Infusion of IL-1 $\beta$  at a dose of 4.0  $\mu$ g/day induced clear signs of physical discomfort to the animals, including piloerection and decreased physical activity, starting a few hours after implantation of the pumps. This visually observed uneasiness gradually diminished during the first day of the treatment period and had disappeared at the end of day 1. Infusion with a dose of 2.0  $\mu$ g IL-1/day also induced signs of discomfort, which were less pronounced than those induced by the 4.0- $\mu$ g IL-1 dose. Treatment of rats with 0.5  $\mu$ g IL-1/day or physiological saline did not perceptibly distress the animals.

#### Effects of IL-1 on plasma ACTH and B and on pituitary ACTH content and adrenal weight

Using osmotic minipumps, rats were continuously infused for 7 days with 0.5, 2.0, or 4.0  $\mu$ g IL-1/day or with physiological saline, and plasma levels of ACTH and B were monitored for 8 days, starting on the morning before implantation of the pumps. Plasma ACTH and B values in animals implanted with saline-filled osmotic pumps varied between 15–51 pg/ml and 24–50 nmol/liter, respectively. Under the present conditions, the operation and infusion procedures, as such, virtually did not alter plasma ACTH and B levels, as demonstrated by the stable hormone levels in saline-treated rats.

Administration of 0.5  $\mu$ g IL-1/day did not affect plasma ACTH and B levels (Fig. 1). Infusion of 2.0  $\mu$ g



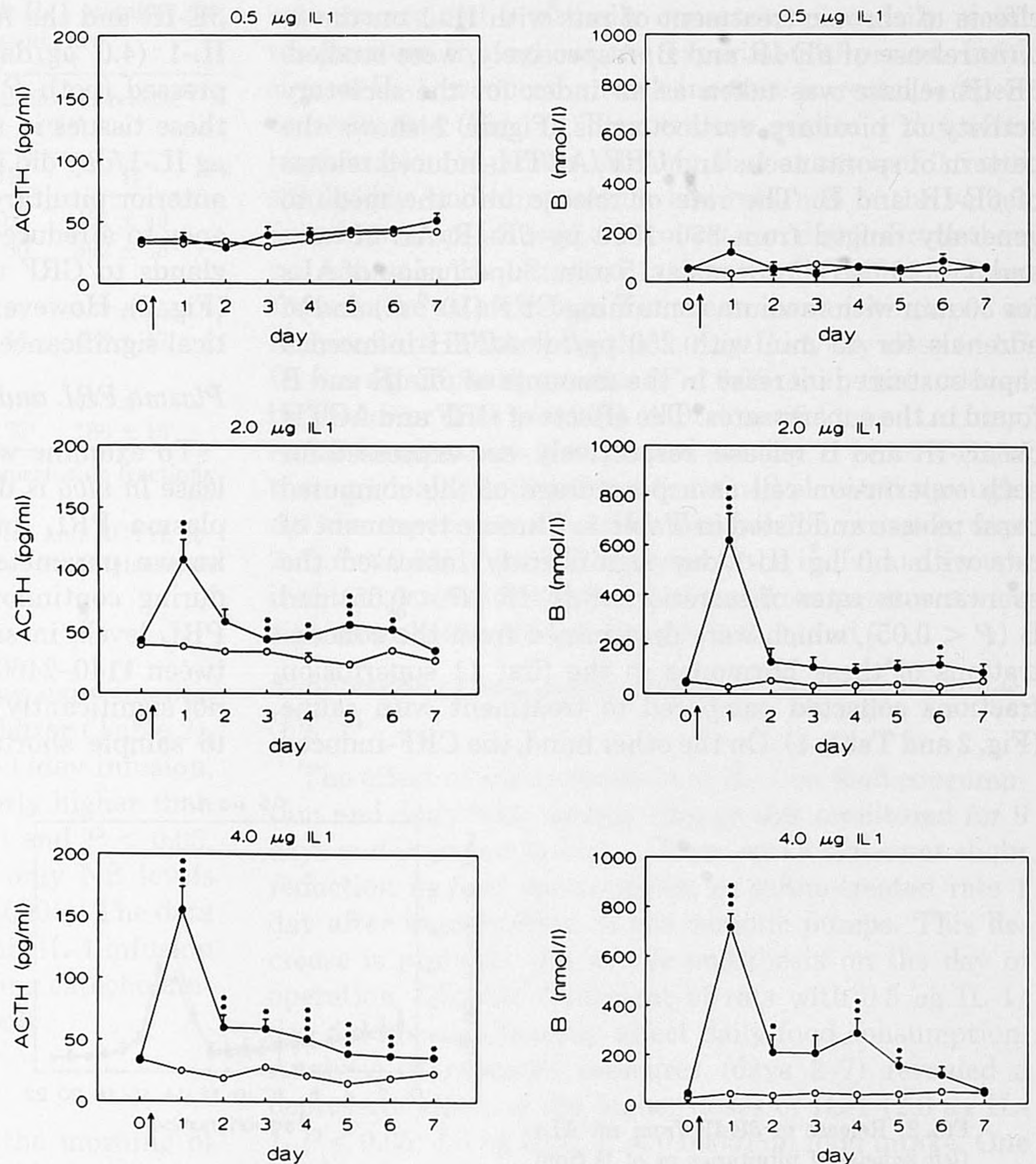


FIG. 1. Effects of continuous infusion for 1 week of different doses of IL-1 (0.5, 2.0, and 4.0  $\mu\text{g}$  IL-1/day; ●) or physiological saline (○) in rats on the concentrations of ACTH (left panels) and B (right panels) in plasma. Osmotic minipumps were implanted between 1400–1600 h on day 0 (arrow). Data are expressed as the mean  $\pm$  SE of five to seven rats. Asterisks mark statistically significant differences between IL-1- and saline-treated rats. \*,  $P < 0.05$ ; \*\*,  $P < 0.005$ ; \*\*\*,  $P < 0.0005$  (by Student's  $t$  test).

IL-1/day induced a significant increase in plasma ACTH and B levels compared to those in saline-treated controls. Repeated measures analysis revealed a significant treatment effect on plasma ACTH (days 2–7;  $P < 0.0005$ ) and B (days 2–7;  $P < 0.05$ ) in IL-1-infused rats compared to saline-treated controls. The maximal effect [ACTH,  $+184 \pm 38\%$  ( $P < 0.005$ ); B,  $+2114 \pm 381\%$  ( $P < 0.0005$ )] was observed on day 1 of treatment. Plasma levels had declined on day 2, but sustained elevations of ACTH and B levels were found up to day 6. *Post-hoc* analysis of individual groups by means of Student's  $t$  test showed a significant increase in plasma levels of ACTH on days 1, 3, 4, 5, and 6 and of B on days 1 and 6 compared to saline-treated control values. When IL-1 was infused at a rate of 4.0  $\mu\text{g}$ /day, a more pronounced effect on plasma ACTH (repeated measures analysis from days 2–7;  $P < 0.0005$ ) and B (days 2–7;  $P < 0.005$ ) levels was observed. Peak levels on day 1 were about 6.2 (ACTH;  $P < 0.0005$ ) and 17.5 (B;  $P < 0.0005$ ) times higher than those in saline-treated rats. Significant elevations of plasma ACTH levels were found from day 1 up to day 7, and

significant elevations of B occurred on days 1, 2, 4, and 5.

The effects of continuous treatment of rats with IL-1 on the ACTH content of the anterior pituitary (2.0- $\mu\text{g}$  IL-1 experiment) and the weight of the adrenal glands (0.5-, 2.0-, and 4.0- $\mu\text{g}$  IL-1/day experiments) were also studied. The ALs of rats treated for 1 week with 2.0  $\mu\text{g}$  IL-1/day contained significantly more immunoreactive ACTH than those of saline-treated rats (IL-1 *vs.* saline,  $436 \pm 45$  *vs.*  $291 \pm 30$  ng;  $P < 0.05$ ). Chronic administration of 2.0 and 4.0  $\mu\text{g}$  IL-1 induced significant increases in total adrenal weight [summated weight of both adrenals of each rat, 2.0  $\mu\text{g}$  IL-1 *vs.* saline,  $46.6 \pm 1.4$  *vs.*  $40.1 \pm 1.6$  mg ( $P < 0.05$ ); 4.0  $\mu\text{g}$  IL-1 *vs.* saline,  $48.7 \pm 1.9$  *vs.*  $44.0 \pm 0.6$  mg ( $P < 0.05$ )]. IL-1 at a dose of 0.5  $\mu\text{g}$ /day failed to affect adrenal weight.

#### Effects of IL-1 treatment on *in vitro* $\beta\text{E-IR}$ and B release (Fig. 2)

After decapitation and dissection, ALs and adrenals were transferred to a superfusion apparatus, and the



effects of chronic treatment of rats with IL-1 on the *in vitro* release of  $\beta$ E-IR and B, respectively, were studied.  $\beta$ E-IR release was taken as an index for the secretory activity of pituitary corticotrophs. Figure 2 shows the pattern of spontaneous and CRF/ACTH-induced release of  $\beta$ E-IR and B. The rate of release into the medium generally ranged from 350–1500 pg  $\beta$ E-IR/AL·15 min and 35–120 ng B/2 adrenals·15 min. Superfusion of ALs for 30 min with medium containing CRF ( $10^{-9}$  M) and of adrenals for 60 min with 250 pg/ml ACTH induced a rapid sustained increase in the amounts of  $\beta$ E-IR and B found in the superfusates. The effects of CRF and ACTH on  $\beta$ E-IR and B release, respectively, are expressed for each superfusion cell as a percentage of the computed basal release and listed in Table 1. Chronic treatment of rats with 4.0  $\mu$ g IL-1/day significantly increased the spontaneous rates of secretion of  $\beta$ E-IR ( $P < 0.05$ ) and B ( $P < 0.05$ ), which were determined from the concentrations of these hormones in the first 11 superfusion fractions collected compared to treatment with saline (Fig. 2 and Table 1). On the other hand, the CRF-induced

$\beta$ E-IR and the ACTH-induced B release from tissue of IL-1 (4.0  $\mu$ g/day)-treated rats were significantly depressed (both  $P < 0.05$ ) compared to the responses of these tissues in saline rats. Treatment with 2.0 and 0.5  $\mu$ g IL-1/day did not affect the basal secretory rate of the anterior pituitary or the adrenal gland, whereas a tendency to a reduced response of the pituitary and adrenal glands to CRF and ACTH, respectively, was observed (Fig. 2). However, these differences did not reach statistical significance (Table 1).

#### Plasma PRL and catecholamine levels

To examine whether IL-1-induced ACTH and B release *in vivo* is due to a stress effect of the IL infusion, plasma PRL and catecholamine concentrations, well known parameters for stress effects, were determined during continuous IL-1 administration. Basal plasma PRL levels in saline- and IL-1-treated rats varied between 1140–2460 pg/ml. Chronic infusion with IL-1 did not significantly affect PRL levels (data not shown). Due to sample shortage, plasma E and NE could only be

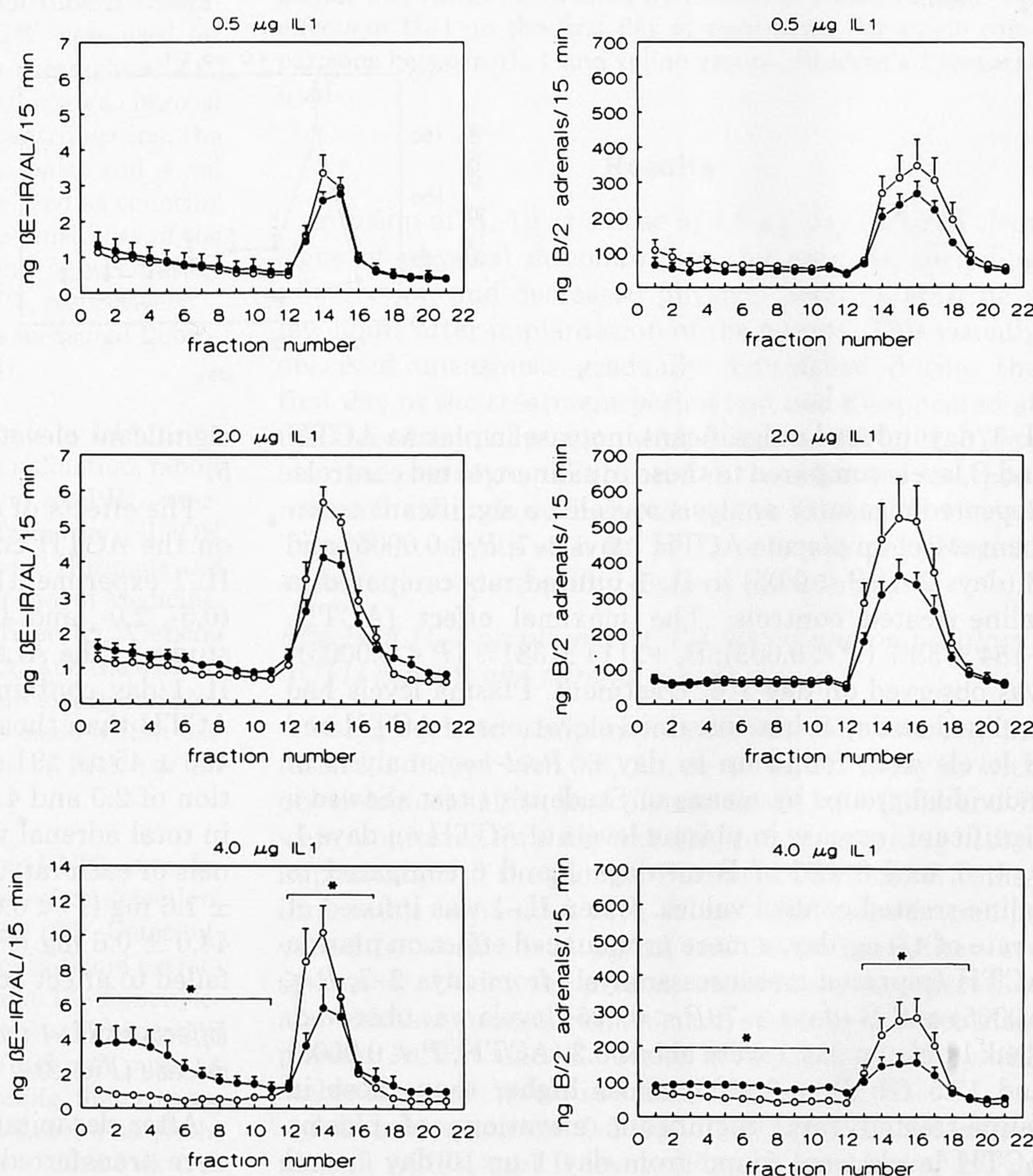


FIG. 2. Release of  $\beta$ E-IR from rat ALs (left panels) of pituitaries or of B from adrenal glands (right panels) superfused *in vitro*. ALs and adrenals from rats chronically treated with IL-1 (●) or saline (○) *in vivo* were superfused for 165 min with medium (fractions 1–11). Subsequently, the ALs were superfused for 30 min (fractions 12–13) with medium containing CRF ( $10^{-9}$  M), and the adrenals for 60 min (fractions 12–15) with medium containing 250 pg/ml ACTH. Thereafter, superfusion was continued with initial medium. Each value represents the mean  $\pm$  SE of six rats. \*, Statistically significant difference from control ( $P < 0.05$ , by Student's *t* test).



TABLE 1. Effects of *in vivo* treatment of rats with IL-1 or saline on basal and CRF/ACTH-induced  $\beta$ E-IR and B release *in vitro*

	Spontaneous release <sup>a</sup>		Stimulation of release (%) <sup>b</sup>	
	$\beta$ E-IR (ng)	B (ng)	$\beta$ E-IR	B
Saline	8.49 $\pm$ 1.56	757 $\pm$ 133	443 $\pm$ 69	401 $\pm$ 49
IL1 (0.5 $\mu$ g)	9.69 $\pm$ 3.26	536 $\pm$ 92	425 $\pm$ 80	362 $\pm$ 35
Saline	10.59 $\pm$ 1.46	745 $\pm$ 64	463 $\pm$ 54	553 $\pm$ 75
IL1 (2.0 $\mu$ g)	13.43 $\pm$ 1.64	647 $\pm$ 78	302 $\pm$ 55	434 $\pm$ 57
Saline	7.25 $\pm$ 0.80	584 $\pm$ 29	840 $\pm$ 166	485 $\pm$ 117
IL1 (4.0 $\mu$ g)	27.78 $\pm$ 6.24 <sup>c</sup>	951 $\pm$ 126 <sup>c</sup>	324 $\pm$ 73 <sup>c</sup>	186 $\pm$ 15 <sup>c</sup>

<sup>a</sup> Total content of  $\beta$ E-IR and B in the first 11 superfusion fractions collected.

<sup>b</sup> Stimulation of  $\beta$ E-IR/B release induced by CRF and ACTH, respectively, is expressed as a percentage of calculated basal release, as described in the text.

<sup>c</sup>  $P < 0.05$  vs. control.

measured in the 2.0- and 4.0- $\mu$ g IL-1/day experiments, with the exception of basal levels in the latter (Table 2). One day after the start of the 2.0- $\mu$ g IL-1/day infusion, plasma E and NE levels were significantly higher than those in the saline animals ( $P < 0.0005$  and  $P < 0.05$ , respectively). In the 4.0- $\mu$ g experiment only NE levels were significantly higher on day 1 ( $P < 0.0005$ ). The data in Table 2 allow us to conclude that chronic IL-1 infusion led to a short-lasting increase in circulating catecholamine levels on the first day of treatment only.

#### Rectal temperature changes (Fig. 3)

Rectal temperature levels of rats on the morning of the day of implantation of the pumps (day 0) varied between 37.1–37.8 C and did not differ significantly between the treatment groups. In addition, saline-treated

rats maintained a virtually constant mean daily rectal temperature throughout the experimental period. Infusion of IL-1 produced a brisk increase in rectal temperature in rats (Fig. 3). Temperature peaked 1 day after implantation of pumps infusing IL-1 at rates of 0.5 and 2.0  $\mu$ g/day, and temperature had returned to the values found in saline-infused control rats on day 3. From then on, values in IL-1-treated rats were not different from those in the saline group. The mean maximal increase in temperature induced by the 2.0- $\mu$ g IL-1/day dose (+1.7 C) was significantly greater ( $P < 0.05$ ) than that induced by the lower dose (+0.9 C). At a dose of 4.0  $\mu$ g IL-1/day, rectal temperature also peaked on day 1 (+1.3 C) and then gradually declined but remained elevated throughout the experimental period (analysis of variance, days 2–7,  $P < 0.05$ ). *Post-hoc* analysis of the individual groups in this experiment revealed that rectal temperatures were significantly elevated on days 1, 2, 3, 4, and 7.

#### Suppression of food intake and daily body weight change (Fig. 4)

The effect of administration of IL-1 on food consumption and daily body weight change was monitored for 9 days and is shown in Fig. 4. There was a transient slight reduction in food consumption in saline-treated rats 1 day after implantation of the osmotic pumps. This decrease is probably due to the anesthesia on the day of operation. Chronic treatment of rats with 0.5  $\mu$ g IL-1/day did not significantly affect daily food consumption. Analysis of repeated measures (days 2–7) revealed a depressive effect of the higher doses of IL-1 (2.0  $\mu$ g IL-1,  $P < 0.05$ ; 4.0  $\mu$ g IL-1,  $P < 0.0005$ ) on food intake. One day after implantation of the pumps, the decrease in food intake was maximal [2.0  $\mu$ g IL-1,  $-7.2 \pm 1.6$  g (vs. saline,  $P < 0.05$ ); 4.0  $\mu$ g IL-1,  $-8.2 \pm 0.9$  g ( $P < 0.0005$ )] and

TABLE 2. Effects of chronic infusion of rats with IL-1 or saline on plasma E and NE concentrations (picograms per ml)

	Day						
	0	1	2	3	4	5	6
E							
Saline	34 $\pm$ 9	9 $\pm$ 2	17 $\pm$ 5	17 $\pm$ 1	20 $\pm$ 5	16 $\pm$ 1	28 $\pm$ 14
IL-1 2 $\mu$ g	21 $\pm$ 7	62 $\pm$ 10 <sup>b</sup>	34 $\pm$ 8	28 $\pm$ 7	20 $\pm$ 11	19 $\pm$ 3	19 $\pm$ 5
NE							
Saline	182 $\pm$ 24	165 $\pm$ 36	202 $\pm$ 36	151 $\pm$ 13	106 $\pm$ 17	191 $\pm$ 70	244 $\pm$ 73
IL-1 2 $\mu$ g	133 $\pm$ 12	323 $\pm$ 51 <sup>a</sup>	260 $\pm$ 52	97 $\pm$ 24	50 $\pm$ 23	121 $\pm$ 39	125 $\pm$ 55
E							
Saline		31 $\pm$ 13	38 $\pm$ 9	20 $\pm$ 4	19 $\pm$ 4	32 $\pm$ 6	16 $\pm$ 4
IL-1 4 $\mu$ g		51 $\pm$ 8	67 $\pm$ 27	19 $\pm$ 9	24 $\pm$ 6	28 $\pm$ 2	11 $\pm$ 3
NE							
Saline		136 $\pm$ 25	134 $\pm$ 24	123 $\pm$ 18	88 $\pm$ 4	72 $\pm$ 18	49 $\pm$ 14
IL-1 4 $\mu$ g		368 $\pm$ 17 <sup>b</sup>	201 $\pm$ 36	54 $\pm$ 12	97 $\pm$ 43	96 $\pm$ 33	76 $\pm$ 36

Analysis of variance from days 2–6 revealed no statistically significant differences for either E or NE levels between rats infused with IL-1 or saline ( $P > 0.10$ ).

<sup>a</sup>  $P < 0.05$  vs. control (by Student's *t* test).

<sup>b</sup>  $P < 0.0005$  vs. control (by Student's *t* test).



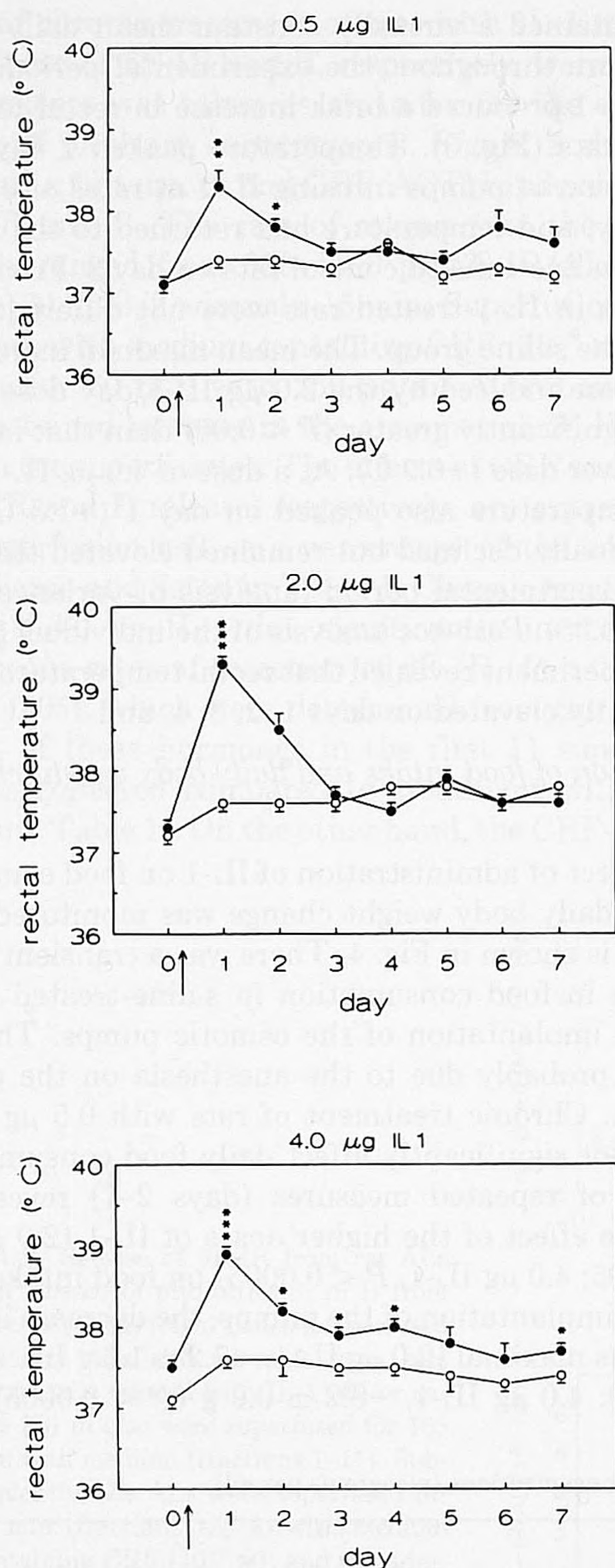


FIG. 3. Effects of chronic administration of IL-1 (0.5, 2.0, and 4.0 µg/day; ●) on rectal temperature compared with values during saline infusion (○). Each point represents the mean  $\pm$  SE of five to seven rats. By analysis of variance, days 2–7: 0.5 µg IL-1/day,  $P < 0.05$ ; 2.0 µg IL-1/day,  $P > 0.10$ ; 4.0 µg/day,  $P < 0.05$ . Statistically significant differences: \*,  $P < 0.05$ ; \*\*,  $P < 0.005$ ; \*\*\*,  $P < 0.0005$  (by Student's *t* test).

values remained significantly lower in these animals than in their saline-treated controls until day 3 (2.0 µg) and day 5 (4.0 µg), respectively.

There was only a small (0.5 µg IL-1,  $-3.4 \pm 1.8$  g; 2.0 µg IL-1,  $-7.1 \pm 1.9$  g) or no (4.0 µg IL-1) decrease in body weight on the first day of saline infusion (Fig. 4). A statistically significant reduction in body weight gain of animals infused with 0.5 µg IL-1/day was found on day 1 ( $-6.3 \pm 1.6$  g; *vs.* saline,  $P < 0.05$ ). Thereafter, IL-

1 treatment did not notably affect the change in body weight of the animals compared to saline infusion. Continuous infusion of 2.0 and 4.0 µg IL-1/day produced a more distinct weight loss in rats. Weight loss was already maximal on the first day of infusion [(2.0 µg IL-1/day,  $-15.2 \pm 1.3$  g (*vs.* saline,  $P < 0.0005$ ); 4.0 µg IL-1/day,  $-11.8 \pm 3.6$  g (*vs.* saline,  $P < 0.005$ )]. The daily body weight change of IL-1-treated rats reached approximately initial levels by the third day. Thereafter, the rate of body weight increase was virtually the same for both IL-1- and saline-treated groups of animals.

Chronic infusion of IL-1 at doses of 0.5, 2.0, and 4.0 µg/day into rats had no effect on total daily fluid intake (data not shown).

## Discussion

The present study demonstrates that continuous ip infusion of rats with rather low doses of IL-1 (2.0 and 4.0 µg/day) induces a dose-dependent and sustained increase in plasma ACTH levels. IL-1 at a dose of 0.5 µg/day was not active. Together with the increased ACTH content of the anterior pituitary, this effect reflects *in vivo* increased biosynthesis of ACTH induced by IL-1. IL-1, indeed, has been reported to increase ACTH biosynthesis in corticotroph cells, possibly at the transcriptional stage (28–30). The changes in plasma ACTH levels are paralleled by those in B; the maximal effect of the IL-1 treatment occurs on day 1, and hormone levels remain on a less elevated plateau level from days 2–6. On day 7, plasma ACTH (2.0-µg experiment) or B (2.0- and 4.0-µg experiments) levels had returned to control values, whereas plasma ACTH in the 4.0-µg IL-1 experiment was still elevated on this day. The increase in plasma B levels was paralleled by an enlargement of the adrenal glands (11–16%), which is a well known consequence of chronic activation of the HPA axis.

There are two other *in vivo* studies on the effects of chronic treatment of rats with IL-1 on the activity of the HPA axis (31, 32). In those studies ACTH and glucocorticoid levels were determined at the end of the treatment period in plasma of decapitated rats. In the present study the effects of continuous infusion of rats with IL-1 on ACTH and B levels in plasma, collected by means of a chronic jugular cannula, were monitored daily for 1 week. In our study, unlike in the above-mentioned studies, plasma PRL, E, and NE levels were followed together with body temperature and food intake to detect aspecific secondary stress effects. Gaillard *et al.* (31) showed in a preliminary report that ACTH levels in plasma of rats chronically treated for 1 week with 5.0 µg IL-1/day and decapitated at the end of the infusion period had not changed, whereas the mean adrenal weight and pituitary ACTH content were significantly increased in IL-1 rats.



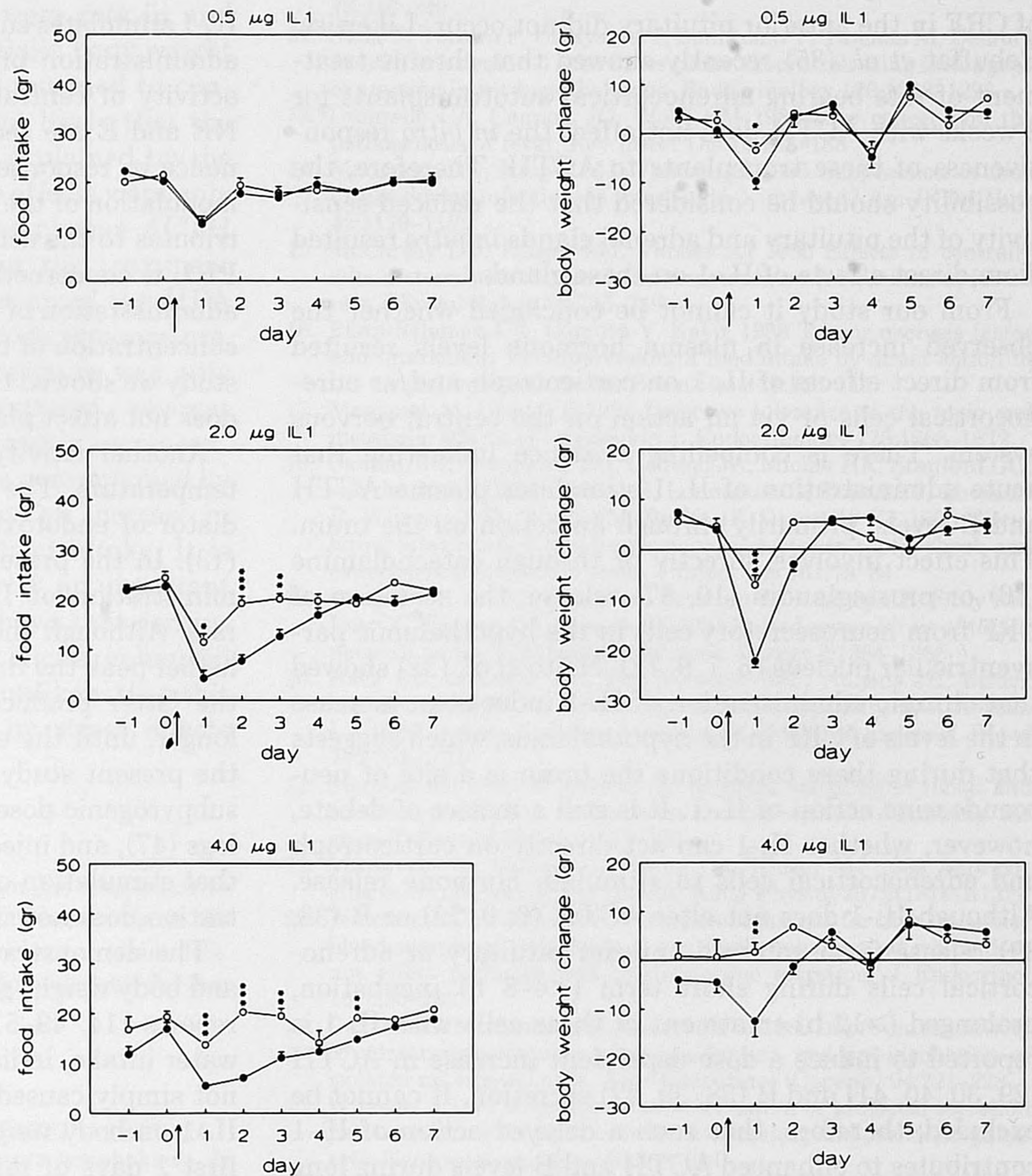


FIG. 4. The effect of continuous infusion of IL-1 (●) or saline (○) for 7 days on food intake and daily body weight change of rats. The daily body weight change for each rat was calculated by subtraction of the daily measured body weights on subsequent days. Statistically significant differences: \*,  $P < 0.05$ ; \*\*,  $P < 0.005$ ; \*\*\*,  $P < 0.0005$  (by Student's  $t$  test).

Naito *et al.* (32), in a cross-sectional study, showed that repetitive administration of  $1.0 \mu\text{g}$  IL-1 twice a day for periods up to 10 days induces an increase in the ACTH content of the anterior pituitary as early as day 3, whereas the wet weight of the adrenal glands had not increased significantly in rats until day 10 of the treatment period. Plasma ACTH concentrations measured 2–4 h after the last bolus injection were significantly higher after 1, 3, 7, and 10 days of treatment with IL-1 compared with those in saline-treated rats.

In our study chronic treatment of rats with  $4.0 \mu\text{g}$  IL-1/day resulted in an increase in the spontaneous *in vitro* release of  $\beta\text{E}$  from the anterior pituitary and of B from the adrenals, whereas no significant effects were observed using lower doses of IL-1. As ACTH and  $\beta\text{E}$  are generally secreted simultaneously (33), one may assume that the secretion of ACTH had also increased. Remarkably, 1 day before decapitation, plasma levels were only slightly, although significantly (ACTH), or not at all (B) elevated compared to values in saline control rats. Thus,

although a firm increase in the *in vitro* secretory activity of the pituitary and adrenal glands was observed, this was only paralleled by a small increase in plasma ACTH levels. One should bear in mind, however, that *in vitro*, these tissues are devoid of any humoral or neuronal regulatory input signal and, thus, might exhibit a secretory pattern that is probably distinct from that *in vivo*. It is difficult to understand why the *in vitro* hormonal responses of the pituitary and adrenal glands of chronically IL-1-treated rats to CRF and ACTH, respectively, were diminished. A depletion of releasable ACTH stores in the pituitary by a higher spontaneous release of ACTH is not likely, as pituitary ACTH levels were significantly increased in IL-1 rats. Alternatively, it might be that long term activation of the HPA axis *in vivo* leads to desensitization of pituitary and adrenal tissues to secretagogues. Continuous intracerebroventricular infusion of CRF in rats for 52 days, however, has been reported to cause a persistent increase in plasma ACTH levels (34), indicating that a significant desensitization to the effect



of CRF in the anterior pituitary did not occur. Likewise, Rebuffat *et al.* (35) recently showed that chronic treatment of rats bearing adrenocortical autotransplants for 2 weeks with ACTH does not affect the *in vitro* responsiveness of these transplants to ACTH. Therefore, the possibility should be considered that the reduced sensitivity of the pituitary and adrenal glands *in vitro* resulted from direct effects of IL-1 on these glands.

From our study it cannot be concluded whether the observed increase in plasma hormone levels resulted from direct effects of IL-1 on corticotroph and/or adrenocortical cells or via an action on the central nervous system. There is compelling evidence indicating that acute administration of IL-1 stimulates plasma ACTH and B levels, primarily through an action on the brain. This effect involves, directly or through catecholamine (36) or prostaglandin (10, 37) release, the secretion of CRF from neurosecretory cells in the hypothalamic paraventricular nucleus (6, 7, 9, 12). Naito *et al.* (32) showed that chronic administration of IL-1 induces an increase in the levels of CRF in the hypothalamus, which suggests that during these conditions the brain is a site of neuroendocrine action of IL-1. It is still a matter of debate, however, whether IL-1 can act directly on corticotroph and adrenocortical cells to stimulate hormone release. Although IL-1 does not alter ACTH (6, 9, 29) or B (38, 39) release from cultured anterior pituitary or adrenocortical cells during short term (<4–8 h) incubation, prolonged (>12 h) treatment of these cells with IL-1 is reported to induce a dose-dependent increase in ACTH (29, 30, 40, 41) and B (38, 39, 42) secretion. It cannot be excluded, therefore, that such a delayed action of IL-1 contributes to enhanced ACTH and B levels during long term treatment *in vivo*. It may be that chronic IL-1 treatment activates the HPA axis via two mechanisms of action: 1) a rapid indirect effect, which increases plasma ACTH and B through activation of the hypothalamic CRF neurons, and 2) a delayed effect, which results from a direct stimulation of pituitary and/or adrenal cells.

Catecholamines released from the sympathetic adrenomedullary system upon stress may contribute to increased plasma ACTH levels (43). Rivier *et al.* (8) showed that acute administration of IL-1 to rats induces a dose-related increase in plasma levels of ACTH, B, E, and NE. We determined plasma levels of NE and E during chronic infusion with IL-1. Two and 4.0  $\mu\text{g}$  IL-1/day induced significant increases in plasma NE and/or E concentrations only on the first day of infusion, whereas a prolonged increase in plasma ACTH levels was found. Thus, although chronic IL-1 administration induces a transient increase in plasma catecholamine levels, activation of pituitary adrenergic receptors by circulating catecholamines is unlikely to be a mechanism by which

IL-1 stimulates sustained ACTH secretion. As peripheral administration of IL-1 has been shown to change the activity of central noradrenergic neurons (36, 44), and NE and E are secreted locally within the median eminence in response to IL-1 (45), we cannot exclude that modulation of the central catecholaminergic system contributes to the action of IL-1 on ACTH levels. As far as PRL is concerned, it has been demonstrated that acute administration of IL-1 to rats does not affect the plasma concentration of this hormone (7, 10, 46). In the present study we showed that chronic administration of IL-1 also does not affect plasma PRL concentrations.

Another activity of IL-1 is the capacity to elevate body temperature. The effects of IL-1 as an endogenous mediator of endotoxin-induced fever are well documented (13). In the present study we confirm that chronic administration of IL-1 induces dose-dependent fever in rats. Although the 2.0- $\mu\text{g}$  dose produced a significantly higher peak the first day of infusion than the 4.0- $\mu\text{g}$  dose, the latter produced a rise in temperature that lasted longer, until the end of the experiment. It is clear from the present study and a number of acute studies using subpyrogenic doses of IL-1 (3), nonpyrogenic IL-1 analogs (47), and injections of IL-1 in the preoptic area (48) that stimulation of ACTH secretion after IL-1 administration does not simply depend on fever production.

The demonstration that IL-1 suppresses food intake and body weight gain is in good agreement with previous reports (14, 49–51). No effects of IL-1 were found on water intake, indicating that the observed weight loss is not simply caused by altered fluid intake. The effect of IL-1 on body weight change was maintained during the first 2 days of infusion and had been lost on day 3 of treatment. An elegant study by Mrosovsky *et al.* (22) clearly demonstrated that recovery of body weight and food intake is not due to failure of the osmotic pumps or hindrance of the release of IL-1 into the peritoneal cavity, and that osmotic pumps are effectively delivering bioactive IL-1 for at least 1 week. These researchers suggested that tolerance to the anorectic effect of IL-1 develops within a few days.

It is not clear whether the anorectic effects of IL-1 are mediated through effects on the brain. Intracerebroventricular injections of IL-1 do not affect food intake (15), supporting a peripheral site of action. However, IL-1 specifically suppresses electrical activity of glucose-sensitive neurons in the hypothalamus (16), and intracerebroventricular injection of anti-CRF antiserum before ip IL-1 administration reduces feeding suppression (51). This suggests that CRF secretion should at least partially be involved in the effect of IL-1 on food intake.

We have shown that continuous ip infusion of rats with IL-1 induces a dose-dependent and sustained activation of the pituitary-adrenal axis. On only the first day



of infusion with 2.0 and 4.0  $\mu\text{g}$  IL-1 were rats ill and plasma catecholamine levels elevated. Daily body weight gain was temporarily reduced, but had returned to control values on day 3. Therefore, it is not likely that the effects on the HPA axis can be solely explained by the illness-inducing effects of IL-1, as these effects were only observed during the first days, whereas plasma ACTH and B levels were elevated throughout the treatment period. It is also not likely that activation of the HPA axis is simply secondary to changes in body temperature, as in the 2.0- $\mu\text{g}$  IL-1 experiment temperature was only elevated during the first 2 days of treatment, whereas plasma ACTH and B levels were persistently increased.

Acute infection and inflammation are accompanied by a sustained activation of the HPA axis, an increase in body temperature, and a decrease in food intake. It is assumed that IL-1 is, directly or indirectly, an important mediator of these changes. Our study shows that continuous administration of IL-1 to rats elicits enhanced activity of the HPA axis and that it is unlikely that this stimulation can be simply explained by stress effects induced by IL-1 treatment.

### Acknowledgments

We would like to thank Dr. L. Swinkels for his assistance in the development of the B assay, and Mr. G. Grutters from the Central Animal Laboratory for expert biotechnical assistance. Mrs. W. van der Velde-van Leeuwen is acknowledged for her analytical support.

### References

- Dinarello CA 1988 Biology of interleukin 1. *FASEB J* 2:108-115
- Dinarello CA, Mier JW 1987 Current concepts lymphokines. *N Engl J Med* 317:940-945
- Besedovsky H, Del Rey A, Sorkin E, Dinarello C 1986 Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science* 233:652-654
- Blalock JE, Smith EM 1985 A complete regulatory loop between the immune and neuroendocrine systems. *Fed Proc* 44:108-111
- Bateman A, Singh A, Kral T, Solomon S 1989 The immune-hypothalamic-pituitary-adrenal axis. *Endocr Rev* 10:92-112
- Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W 1987 Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* 238:522-524
- Uehara A, Gottschall PE, Dahl RR, Arimura A 1987 Interleukin-1 stimulates ACTH release by an indirect action which requires endogenous corticotropin releasing factor. *Endocrinology* 121:1580-1582
- Rivier C, Vale W, Brown M 1989 In the rat, interleukin-1 $\alpha$  and - $\beta$  stimulate adrenocorticotropin and catecholamine release. *Endocrinology* 125:3096-3102
- Berkenbosch F, Van Oers J, Del Rey A, Tilders F, Besedovsky H 1987 Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1. *Science* 238:524-526
- Katsuura G, Gottschall PE, Dahl RR, Arimura A 1988 Adrenocorticotropin release induced by intracerebroventricular injection of recombinant human interleukin-1 in rats: possible involvement of prostaglandin. *Endocrinology* 122:1773-1779
- Ovadia H, Abramsky O, Barak V, Conforti N, Saphier D, Weidenfeld J 1989 Effect of interleukin-1 on adrenocortical activity in intact and hypothalamic deafferented male rats. *Exp Brain Res* 76:246-249
- Suda T, Tozawa F, Ushiyama T, Sumitomo T, Yamada M, Demura H 1990 Interleukin-1 stimulates corticotropin-releasing factor gene expression in rat hypothalamus. *Endocrinology* 126:1223-1228
- Dinarello CA, Cannon JG, Wolff SM 1988 New concept on the pathogenesis of fever. *Rev Infect Dis* 10:168-188
- McCarthy DO, Kluger MJ, Vander AJ 1985 Suppression of food intake during infection. Is interleukin-1 involved? *Am J Clin Nutr* 42:1179-1182
- McCarthy DO, Kluger MJ, Vander AJ 1986 Effects of centrally administered interleukin-1 and endotoxin on food intake of fasted rats. *Physiol Behav* 36:745-749
- Plata-Salaman CR, Oomura Y, Kai Y 1988 Tumor necrosis factor and interleukin-1 $\beta$ : suppression of food intake by direct action in the central nervous system. *Brain Res* 448:106-114
- Mengozzi M, Ghezzi P 1991 Defective tolerance to the toxic and metabolic effects of interleukin 1. *Endocrinology* 128:1668-1672
- Cannon JG, Tompkins RG, Gelfand JA, Michie HR, Stanford GG, van der Meer JWM, Endres S, Lonneman G, Corsetti J, Chernow B, Wilmore DW, Wolff SM, Burke JF, Dinarello CA 1990 Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 161:79-84
- Scuderi P, Lam KS, Ryan KJ, Petersen E, Sterling KE, Finly PR, Ray CG, Slymen DJ, Salmon SE 1986 Raised serum levels of tumor necrosis factor in parasitic infections. *Lancet* 2:1364-1365
- Hack CE, de Groot ER, Felt-Boersma RJF, Nuijens JH, Strack van Schijndel RJM, Eerenberg-Belmer AJM, Thijs LG, Aarden LA 1989 Increased plasma levels of interleukin-6 in sepsis. *Blood* 74:1704-1710
- Steffens AB 1969 A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol Behav* 4:833-836
- Mrosovsky N, Molony LA, Conn CA, Kluger MJ 1989 Anorexic effects of interleukin 1 in the rat. *Am J Physiol* 257:R1315-R1321
- Wiersma J, Castelijns J 1985 A chronic technique for high frequency blood sampling/transfusion in the freely behaving rat which does not affect prolactin and corticosterone secretion. *J Endocrinol* 107:285-292
- Goverde HJM, Pesman GJ, Benraad ThJ 1980 Improved sensitivity to adrenocorticotrophin after purification and pre-incubation of isolated rat adrenal cells. *Acta Endocrinol (Copenh)* 94:221-228
- Wiersma J 1981 A detailed characterization of prolactin secretion patterns during daylight in individual cycling and pseudopregnant rats. *Neuroendocrinology* 33:288-294
- van der Hoorn FAJ, Boomsma F, Man in't Veld AJ, Schalekamp MADH 1989 Determination of catecholamines in human plasma by high performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection. *J Chromatogr* 487:17-28
- Sweep CGJ, Boersma CJC, VM Wiegant 1990 Isoproterenol-stimulated release of  $\beta$ -endorphin and related peptides from the rat pituitary neurointermediate lobe *in vitro*: evidence for preferential release of certain molecular forms of  $\beta$ -endorphin. *Neuropeptides* 17:63-73
- Brown SL, Smith LR, Blalock JE 1987 Interleukin-1 and interleukin-2 enhance proopiomelanocortin gene expression in pituitary cells. *J Immunol* 139:3181-3183
- Fukata J, Usui T, Naitoh Y, Nakai Y, Imura H 1989 Effects of recombinant human interleukin-1 $\alpha$ , -1 $\beta$ , -2 and -6 on ACTH synthesis and release in the mouse pituitary tumour cell line AtT-20. *J Endocrinol* 122:33-39
- Suda T, Tozawa F, Ushiyama T, Tomori N, Sumitomo T, Nakagami Y, Yamada M, Demura H, Shizume K 1989 Effects of protein kinase-C-related adrenocorticotropin secretagogues and interleukin-1 on proopiomelanocortin gene expression in rat anterior pituitary cells. *Endocrinology* 124:1444-1449
- Gaillard RC, Saudan P, Corder R, Muller AF, Effect of chronic administration of interleukin 1 $\beta$  on the corticotroph axis. 23rd Annual Meeting of the European Society for Clinical Investigation, Athens, Greece, 1989, p A8 (Abstract)
- Naito Y, Fukata J, Nakaishi S, Nakai Y, Hirai Y, Tamai S, Mori K, Imura H 1990 Chronic effects of interleukin-1 on hypothalamus,



- pituitary and adrenal glands in rat. *Neuroendocrinology* 51:637-641
33. Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, Bloom F 1977  $\beta$ -Endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science* 197:1367-1369
  34. Gertz BJ, Contreras LN, McComb DJ, Kovacs K, Tyrrell JB, Dallman MF 1987 Chronic administration of corticotropin-releasing factor increases pituitary corticotroph number. *Endocrinology* 120:381-388
  35. Rebuffat P, Neri G, Andreis PG, Belloni AS, Mazzocchi G, Musajo FG, Nussdorfer GG 1991 Effects of prolonged treatment with adrenocorticotropin on the morphology and function of rat adrenocortical autotransplants. *Cytobios* 65:101-113
  36. Weidenfeld J, Abramsky O, Ovadia H 1989 Evidence for the involvement of the central adrenergic system in interleukin 1-induced adrenocortical response. *Neuropharmacology* 28:1411-1414
  37. Katsuura G, Arimura A, Koves K, Gottschall PE 1990 Involvement of organum vasculosum of lamina terminalis and preoptic area in interleukin 1 $\beta$ -induced ACTH release. *Am J Physiol* 258:E163-E171
  38. Salas MA, Evans SW, Levell MJ, Whicher JT 1990 *In vitro* effect of rIL 1 and rIL 6 on corticosterone release by isolated rat adrenal gland cells. In: Dinarello CA (ed) *Molecular and Cellular Biology of Cytokines*. Wiley-Liss, New York, pp 427-432
  39. Tominaga T, Fukata J, Naito Y, Usui T, Murakami N, Fukushima M, Nakai Y, Hirai Y, Imura H 1991 Prostaglandin-dependent *in vitro* stimulation of adrenocortical steroidogenesis by interleukins. *Endocrinology* 128:526-531
  40. Woloski BMRNJ, Smith EM, Meyer WJ, Fuller GM, Blalock JE 1985 Corticotropin-releasing activity of monokines. *Science* 230:1035-1037
  41. Kehrer P, Turnill D, Dayer JM, Muller AF, Gaillard RC 1988 Human recombinant interleukin-1 $\beta$  and - $\alpha$ , but not recombinant tumor necrosis factor- $\alpha$  stimulate ACTH release from rat anterior pituitary cells *in vitro* in a prostaglandin E<sub>2</sub> and cAMP independent manner. *Neuroendocrinology* 48:160-166
  42. Winter JSD, Gow KW, Perry YS, Greenberg AH 1990 A stimulatory effect of interleukin-1 on adrenocortical cortisol secretion mediated by prostaglandins. *Endocrinology* 127:1904-1909
  43. Tilders FJH, Berkenbosch F, Smelik PG 1985 Control of secretion of peptides related to adrenocorticotropin, melanocyte stimulating hormone and endorphin. In: Van Wimersma Greidanus TJB, Lamberts SWJ (eds) *Frontiers in Hormone Research*. Karger, Basel, vol 14:161-196
  44. Dunn AJ 1988 Systemic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism paralleling the increased plasma corticosterone. *Life Sci* 43:429-435
  45. Matta SG, Singh J, Newton R, Sharp BM 1990 The adrenocorticotropin response to interleukin-1 $\beta$  instilled into the rat median eminence depends on the local release of catecholamines. *Endocrinology* 127:2175-2182
  46. Berkenbosch F, de Goeij DEC, Del Rey A, Besedovsky HO 1989 Neuroendocrine, sympathetic and metabolic responses induced by interleukin-1. *Neuroendocrinology* 50:570-576
  47. Naitoh Y, Fukata J, Tamia S, Mori K, Interleukin-1 $\beta$  analogues with markedly reduced pyrogenic activity can stimulate ACTH secretion in conscious, freely moving rats. 71st Annual Meeting of The Endocrine Society, Seattle WA, 1989, p 144 (Abstract)
  48. Morimoto A, Murakami N, Nakamori T, Sakata Y, Watanabe T 1989 Possible involvement of prostaglandin E in development of ACTH response in rats induced by human interleukin-1. *J Physiol (Lond)* 411:245-256
  49. Otterness IG, Golden HW, Brissette WH, Seymour PA, Daumy GO 1989 Effects of continuously administered murine interleukin-1 $\alpha$ : tolerance development and granuloma formation. *Infect Immunol* 57:2742-2750
  50. Hellerstein MK, Meydani SN, Meydani M, Wu K, Dinarello CA 1989 Interleukin-1-induced anorexia in the rat. *J Clin Invest* 84:228-235
  51. Uehara A, Sekiya C, Takasugi Y, Namiki M, Arimura A 1989 Anorexia induced by interleukin 1: involvement of corticotropin-releasing factor. *Am J Physiol* 257:R613-R617